S exigua; (5) chlorfluazuron and tebufenozide and methoxyfenozide in chlorfluazuron-resistant strains of tea tortrix, *H magnamima*; and (6) flufenoxuron and tebufenozide in flufenoxuron-resistant strains of *S exigua and C pomonella*. Attempts to select *S exigua* resistant to tebufenozide in the laboratory have so far been unsuccessful. Adults developing from larvae maintained over 12 generations at LC_{25} levels of tebufenozide had complete loss of fecundity.[14]

Rohm and Haas Company has developed a pro-active platform to manage resistance to ecdysone agonist insecticides. Global resistance monitoring programs are currently underway on *C pomonella*, *S exigua*, *H mamnamima*, certain Heliothine pests, and certain leafroller species to establish baseline susceptibility data as well to detect any shifts in susceptibility over time. In addition, we pro-actively advocate the use of traditional approaches for managing insecticide resistance, including frequent and repeated rotation with other insecticides with different modes of action to minimize selection pressure, limitations on the number and sequences of applications on certain crops, and incorporation of integrated pest management methods to reduce pesticide use and prolong these products in the marketplace.

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Synthesis and insecticidal activity of 3-aminoquinazolinone derivatives

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Abstract: The discovery, structure–activity relationships and insecticidal properties of R-768 (1-propionyl-3-(3-pyridylmethylamino)-1,2,3,4-tetrahydroquinazolin- 2-one) and related compounds are described.

Keywords: 3-aminoquinazalones; R-768; insecticide; aphicide

In the course of research on methoxyacrylate fungicides, it was found that compounds having a carbamate group instead of a methoxyacrylate group in the relevant compounds also showed fungicidal activity against a broad spectrum of fungi.[1] Structural modification of those compounds was conducted to find a more active compound.[2] In the synthetic process, 2-halomethylphenyl carbamates (Fig 1; **1**) were used as one of the starting materials.[3] These proved to be interesting intermediates for synthesizing heterocycles, because they have two different electrophilic functional groups which can react with a primary amine at the 1,1-position of the

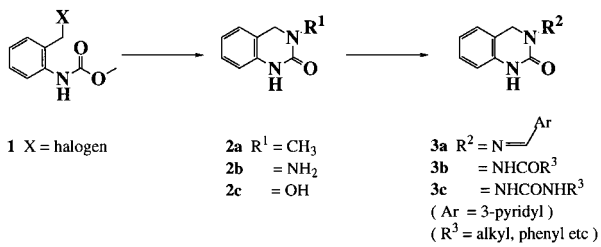

Figure 1. Synthesis of 3-substituted quinazolinones.

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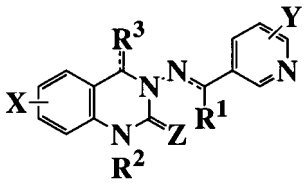
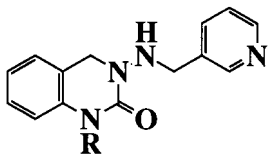


R1 : H > CH_3
R2 : alkyl ≒ alkoxycarbonyl ≦ acyl
R3 : H ≫ alkyl, oxo
X : H ≧ halo ≒ alkyl ≒ alkoxy
Y : H ≫ halogen, alkoxy etc.
Z : O ≒ S
imino form ≒ reduced form

Figure 2. Structure – activity relationship of 3-aminoquinazolinone derivatives.

nucleophlic reactant to give cyclic derivatives. As was expected, they reacted with excess of methylamine on the halomethyl moiety to give first the benzylamino derivative, which was then converted to 3-methylaminoquinazolinone (**2a**) in excellent yield. The Compound **1** also reacted with hydrazine and hydroxylamine to give **2b** and **2c**, respectively. Similar reactions had already been reported in the literature, in which the —NCO group of 2-halomethylphenyl isocyanates reacted with a primary amine to give urea derivatives. The ureas were then cyclized by treatment with base to form the *N*-substituted 2-amino-4*H*-3,1-benzoxazines.[3–5] In our experiments with compound **1**, the benzoxazine derivatives were not detected at all as by-products.

Several insecticides having a semicarbazide group have been reported, *N*-phenylcarbamoylpyrazolines being well known to be a highly active class of insecticide.[6,7] Therefore the 3-amino compound, **2b**, having a semicarbazide moiety, and its derivatives were expected to possess insecticidal activity. Of



4 R = H
R-768, R = propionyl

Figure 3. Structure of the reduced form and R-768.

Table 1. Activity of R-768 against major species of aphid

Chemical	LC_{95} (*mg litre*$^{-1}$)[a]		
	Myzus persicae	*Aphis gossypii*	*Brevicoryne brassicae*
R-768	0.1–0.3	0.1–0.3	0.3–1
Imidacloprid	0.1–0.3	0.01–0.1	0.1–0.3
Acephate	10–30	–[b]	–[b]
Lambda-cyhalothrin	0.1–1	0.1–1	0.1–1

[a] Assessed six days after treatment.
[b] Not Tested.

several derivatives synthesized and tested for insecticidal activity (Fig 1), the imino derivative **3a**, synthesized from **2b** and pyridine-3-aldehyde, was found to show high insecticidal activity against the Hemipterous insect, *Myzus persicae* Sulz, while the amide **3b** and the urea **3c** were inactive. From these preliminary results, we selected **3a** as a lead compound and synthesized analogues to optimize the insecticidal activity.

Figure 2 summarizes the structure–activity relationships of 3-substituted quinazolinones. Among the compounds having the imino moiety, those of the 3-pyridyl azomethine type showed the highest activity. Replacement of 3-pyridyl by substituted phenyl, heterocycles other than 3-pyridyl or alkyl groups decreased the activity. Although the 2-chloropyridylmethyl group was used successfully to generate nitromethylene insecticides,[8] halogeno-substituted 3-pyridyl derivatives in the series of compounds with structure **3a** were markedly less active. Furthermore, any substitution in the pyridine ring failed to increase the activity.

The activity of quinazolinone derivatives with small alkyl groups at the 1-position in the quinazolinone ring was almost equal to that of **3a**, but compounds with long or bulky alkyl substituents showed decreased activity. Derivatives with formyl, lower alkylcarbonyl and lower alkoxycarbonyl groups showed greater activity. The optimum carbon chain length for activity seemed to be two to four. Quinazolinone derivatives with alkyl or oxo at the 4-position were much less active, whereas all quinazolinone derivatives with halogen, alkyl or alkoxy substituents at the 5 to 8 positions showed high activity, differences in activity between them being relatively small. The activity of the 2-thione derivative of **3a** was almost equal to that of **3a** itself.

The imino moiety of **3a** was reduced to give compound **4** (Fig 3). This reduced derivative possessed almost the same activity as **3a** in a greenhouse test. However, **3a** did not show good activity in field trials, whereas the reduced derivative **4** showed excellent activity. It was found that the poor performance of compound **3a** in the field was due to photo-instability, its half-life being <1 h in a sunlight test, while that of **4** was >8 h under the same conditions. Because compound **4** was sufficiently stable to show insecticidal activity in field trials, optimization of the structure and activity was progressed using the reduced form. The effects of substituents R^1, R^2, R^3, X, Y and Z (Fig 2) on the activity of the reduced form were almost the same as in the imino form. Quinazolinone derivatives containing lower alkyl and lower alkoxycarbonyl substituents at the 1-position in the quinazolinone ring were more active than **4**, and it was thought that the substituents at the 1-position in the quinazolinone ring play an important role in promoting the penetration of the compound through the cuticular layer of insects. The quinazolinone derivative with a pro-

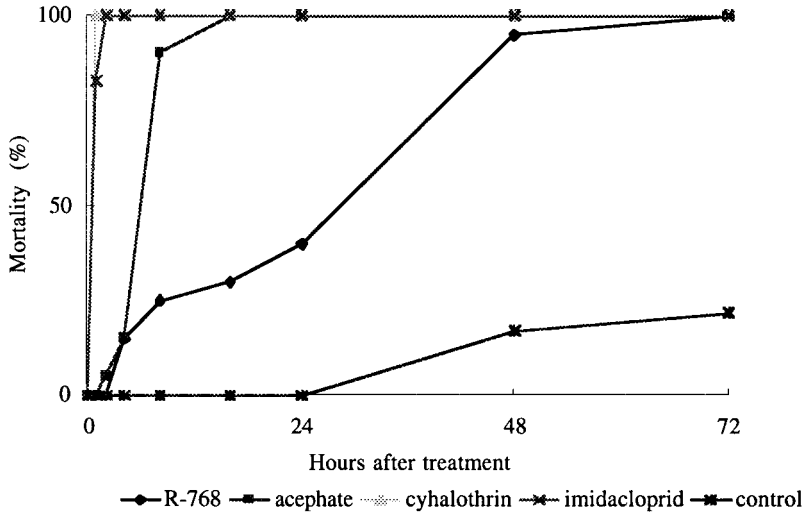


Figure 4. Time-course of mortalities of *Myzus persicae* after spray. Stage: Apterous adult. Diet: Cabbage leaf disc, 80 mm diameter on agar. Treatment: Spraying with 20 ml water solution of compound (100 mg litre^{-1}) to 20 insects on the leaf disc

pionyl substituent at the 1-position showed the greatest activity. As a result, R-768 (Fig 3) was selected as a candidate for development, taking into account its insecticidal activity and its physicochemical properties, which would influence the formulation.

Although R-768 had no effect on Lepidopterous and Coleopterous insects and mites, it showed excellent activities against some Hemipterous insect pests. Its activities against *M persicae and Aphis gossypii* Glov were almost equal to those of imidacloprid, and superior to those of acephate (Table 1).

The lethal action of R-768 against *M persicae* was slower than that of the conventional insecticides in that aphids sprayed with R-768 needed a few days to die (Fig 4). These affected aphids immediately stopped feeding and abandoned the sprayed leaf. The inhibition of feeding of these affected aphids

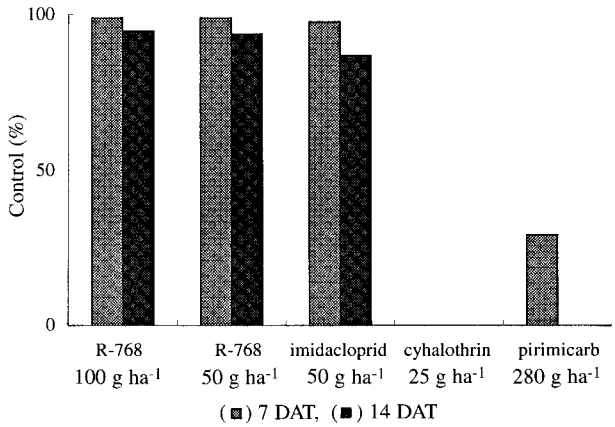


Figure 5. Efficacy of R-768 against *Aphis gossypii* on potato in a field trial. Application: 7 August 1998 in Japan. Spray: 500 litre ha^{-1}.

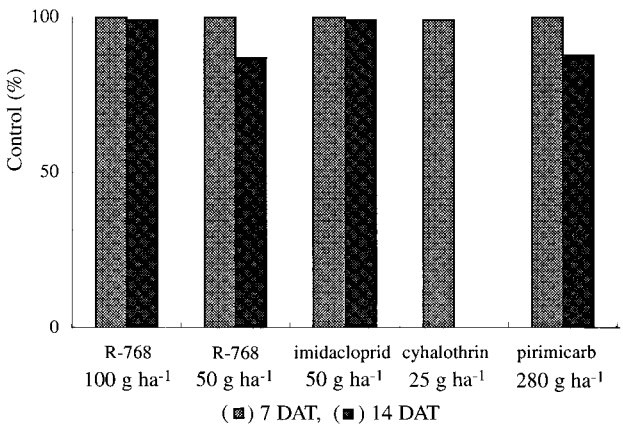


Figure 6. Efficacy of R-768 against *Rhopalosiphum padi* on wheat in a field trial. Application: 17 July 1998 in Japan. Spray: 400 litre ha^{-1}.

Table 2. Activity of R-768 against resistant strains of aphid

Species	*Strain*	LC_{50} (mg litre^{-1})[a]
Myzus persicae	US1L (susceptible standard)	0.8–0.9
	794J (resistant to OPs, carbamates and pyrethroids)	0.5
	926B (tolerant to imidacloprid)	0.8
Aphis gossypii	171B (susceptible standard)	0.7
	968E (resistant to OPs, carbamates and pyrethroids)	0.2

[a] Assessed six days after treatment.

seemed to be so strong that they never regained the ability to suck even after they were transferred to untreated plants. Although they produced a few offspring before death, the offspring were also unable to feed and died without growing. These actions of R-768 are different from the conventional insecticides, suggesting that R-768 has a novel mode of action. In the laboratory study, R-768 was equally effective against organophosphate-, carbamate- and pyrethroid-resistant strains of aphid as well as against susceptible strains of *M persicae* and *A gossypii* (Table 2). This biological performance is also consistent with a new mode of action.

In a field trial on potatoes, the efficacy of R-768 at 50 or 100 g ha^{-1} against the strain of *A gossypii* that was resistant to lambda-cyhalothrin and pirimicarb, was comparable to that of imidacloprid at 50 g ha^{-1} (Fig 5). These results indicated that R-768 possesses no cross-resistance to other insecticides and it will be useful as a candidate in areas where resistance problems become more serious. R-768 aso showed excellent control of aphids on wheat (Fig 6), on apple trees (Fig 7) and on various crops, without phytotoxicity, in the field trials.

On the other hand, R-768 was safe to beneficial insects such as *Bombyx mori* L, *Osmia cornifrons*, *Bombus terrestris* and *Apis mellifera* L, and nontarget arthropods such as *Harmonia axyridis*, *Chrysopa nipponensis*, *Orius* sp, *Aphelinus* sp, *Ephedrus japonicus* and *Misumenops tricuspidatus* in laboratory studies. This outstanding selectivity was also demonstrated for the pollinators of tomato and strawberry in a greenhouse study, and for some natural enemies in the field trials. Therefore R-768 could be one of a few aphid control agents which can be useful soon after releasing the pollinators and natural enemies in biological control systems.

In conclusion, R-768 is a representative of a new and highly active class of aphid control agent with large margins of safety to beneficial insects and natural enemies. Lack of cross-resistance indicates that R-768 could be useful as a candidate in the resistant management strategies. Its favorable characters make it especially suitable for IPM programmes.

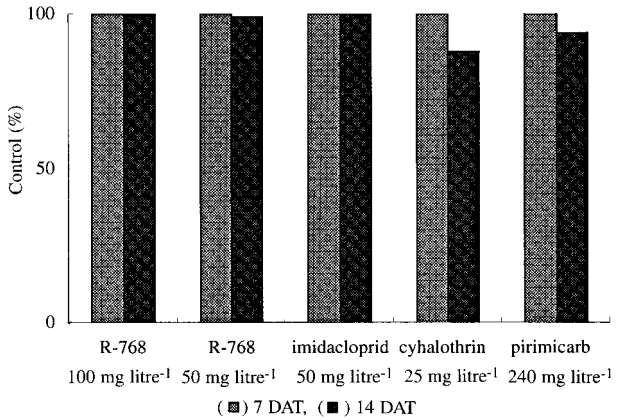


Figure 7. Efficacy of R-768 against *Aphis citricola* on apple trees in a field trial. Application: 22 May 1998 in Japan. Spray: 1500 litre ha^{-1}.

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Expression of *Neurospora crassa* β-tubulin, target protein of benzimidazole fungicides, in *Escherichia coli*

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Abstract: β-Tubulin of a wild-type *Neurospora crassa* strain was expressed using pET-16b, pET-29a(+), and pET-32a(+) expression vectors in *Escherichia coli* BL21 (DE3) strain. Yield of the expressed soluble protein was estimated to be about 0.1 mg ml^{-1} culture broth. The β-tubulins with S-Tag expressed by pET-29a(+) and pET-32a(+) bound to the S-protein Agarose by affinity binding but the thrombin and enterokinase treatments did not release β-tubulin, suggesting that the protease cleavage sites connecting S-Tag and β-tubulin were not exposed to approach of the proteases. The β-tubulin expressed by pET-16b did not bind to nickel resin, suggesting that its His-Tag was folded into the protein core. The protein expressed by pET-32a(+) was bound to the nickel resin and purified by the column chromatography.

Keywords: β-tubulin; gene expression; *Neurospora crassa: Escherichia coli;* expression vector

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